INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/11865

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :A61K 38/48; B01L 11/00; C12N 9/74; C12Q 1/56; G01N 33/00
US CL :422/73, 101; 424/94.64; 435/13, 214
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 422/73, 101; 424/94.64; 435/13, 214

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST, CHEMICAL ABSTRACTS, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96/31245 A1 (HAMOLTON CIVIC HOSPITALS RESEARCH DEVELOPEMENT, INC.) 10 October 1996, see page 7 first full paragraph.	1-27
X	US 5,643,192 A (HIRSH et al.) 01 July 1997, see column 4 Example.	1-27
X	US 5,795,780 A (CEDERHOLM-WILLIAMS et al.) 18 August 1998, see column 5.	1-27
A, P	WO 99/45938 A1 (BIOSURGICAL CORPORATION) 16 September 1999,see entire document.	1-27
A, P	US 6,063,297 A (ANTANAVICH et al.) 16 May 2000, see entire document.	1-27
A	US 5,510,102 A (COCHRUM) 23 April 1996, see entire document.	1-27

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 09 AUGUST 2000	Date of mailing of the international search report 28 AUG 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer RALPH GITOMER Telephone No. (703) 308-1235

Form PCT/ISA/210 (second sheet) (July 1998)*

PA . .NT COOPERATION TREAT

From the iNTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2 5C24
Arlington, VA 22202
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n its capacity as elected Office

Date of mailing (day month year) 29 March 2001 (29.03.01)	
International application No. PCT US00 11865	**Applicant's or agent's file reference** 30088-pct
International filing date (day month year) 02 June 2000 (02.06.00)	**Priority date** (day month year) 04 June 1999 (04.06.99)
Applicant COELHO, Philip, H. et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

04 January 2001 (04.01.01)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

S. Mafla

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PATENT COOPERATION TREATY

REC'D 21 AUG 2001

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PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

14

Applicant's or agent's file reference 30088-pct	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US00/11865	International filing date *(day/month/year)* 02 JUNE 2000	Priority date *(day/month/year)* 04 JUNE 1999
International Patent Classification (IPC) or national classification and IPC Please See Supplemental Sheet.		
Applicant THERMOGENESIS CORP.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets.

   [1] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 44 sheets.

3. This report contains indications relating to the following items:

   I [X] Basis of the report

   II [ ] Priority

   III [ ] Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV [ ] Lack of unity of invention

   V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI [ ] Certain documents cited

   VII [ ] Certain defects in the international application

   VIII [ ] Certain observations on the international application

Date of submission of the demand	Date of completion of this report
04 JANUARY 2001	03 JULY 2001
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer RALPH GITOMER Telephone No. (703) 308-1235

Form PCT/IPEA/409 (cover sheet) (July 1998)*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/11865

I. Basis of the report

1. With regard to the elements of the international application:*

[ ] the international application as originally filed

[X] the description:
pages (See Attached) , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

[X] the claims:
pages (See Attached) , as originally filed
pages _____ , as amended (together with any statement) under Article 19
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

[X] the drawings:
pages (See Attached) , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

[X] the sequence listing part of the description:
pages (See Attached) , as originally filed
pages _____ , filed with the demand
pages _____ , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

5. [ ] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

*** Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/11865

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	(Please See supplemental sheet)	YES
	Claims	(Please See supplemental sheet)	NO
Inventive Step (IS)	Claims	(Please See supplemental sheet)	YES
	Claims	(Please See supplemental sheet)	NO
Industrial Applicability (IA)	Claims	(Please See supplemental sheet)	YES
	Claims	(Please See supplemental sheet)	NO

2. citations and explanations (Rule 70.7)

Claims 1, 2, 4-9, 18-19, 21, 26-51, 53 lack novelty under PCT Article 33(2) as being anticipated by Cederholm-Williams.

Cederholm-Williams (5,795,780) entitled "Method of Use of Autologous Thrombin Blood Fraction in a Cell Culture with Keratinocytes" teaches in column 2 last 2 paragraphs, a thrombin blood fraction can be prepared in about one or two hours from whole blood and used in an autologous medical procedure. In column 4 lines 28-36, the thrombin can be stored for a period of months or days. In column 5 first full paragraph, whole blood is obtained, plasma is fractionated by any separation technique. In column 5 last 2 paragraphs, saline, buffers and other compounds may be added. Calcium chloride is added to convert prothrombin to thrombin. In column 6 line 54, the thrombin can be prepared in only about 45 minutes. In column 9 last full paragraph, a syringe is shown. In column 12 first full paragraph, the dose of the thrombin depends upon its use. In column 18 claim 2 the thrombin concentration can vary from 1-2000 units.

All the claimed features are taught by Cederholm-Williams for the same function as claimed. Note that changing the concentration of the thrombin by diluting with various physiologically acceptable solutions is taught by Cederholm-Williams as well as to employ a desired quantity of thrombin solution.

Claims 3, 10-17, 20, 22-25, 51, 52 lack an inventive step under PCT Article 33(3) as being obvious over each of Cederholm-Williams and Hirsh.

Cederholm-Williams (5,795,780) entitled "Method of Use of Autologous Thrombin Blood Fraction in a Cell Culture with Keratinocytes" teaches in column 2 last 2 paragraphs, a thrombin blood fraction can be prepared in about one or two hours from whole blood and used in an autologous medical procedure. In column 4 lines 28-36, the thrombin can be stored for a period of (Continued on Supplemental Sheet.)

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

CLASSIFICATION:
The International Patent Classification (IPC) and/or the National classification are as listed below:
IPC(7): A61K 38/48; B01L 11/00; C12N 9/74; C12Q 1/56; G01N 33/00 and US Cl.: 422/73, 101; 424/94.64; 435/13, 214

I. BASIS OF REPORT:

This report has been drawn on the basis of the description,
page(s) 1, as originally filed.
page(s) 2-18, filed with the demand.
and additional amendments:
NONE

This report has been drawn on the basis of the claims,
page(s) none, as originally filed.
page(s) none, as amended under Article 19.
page(s) 19-25, filed with the demand.
and additional amendments:
NONE

This report has been drawn on the basis of the drawings,
page(s) 1-15, as originally filed.
page(s) none, filed with the demand.
and additional amendments:
none

This report has been drawn on the basis of the sequence listing part of the description:
page(s) NONE, as originally filed.
pages(s) NONE, filed with the demand.
and additional amendments:
NONE

V. 1. REASONED STATEMENTS:
The report as to Novelty was positive (YES) with respect to claims 3, 10-17, 20, 22-25, 51, 52.
The report as to Novelty was negative (NO) with respect to claims 1, 2, 4-9, 18-19, 21, 26-50, 53.
The report as to Inventive Step was positive (YES) with respect to claims none.
The report as to Inventive Step was negative (NO) with respect to claims 1-53.
The report as to Industrial Applicability was positive (YES) with respect to claims 1-53.
The report as to Industrial Applicability was negative (NO) with respect to claims none.

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):
months or days. In column 5 first full paragraph, whole blood is obtained, plasma is fractionated by any separation technique. In column 5 last 2 paragraphs, saline, buffers and other compounds may be added. Calcium chloride is added to convert prothrombin to thrombin. In column 6 line 54, the thrombin can be prepared in only about 45 minutes. In column 9 last full paragraph, a syringe is shown.

Hirsh (5,643,192) entitled "Autologous Fibrin Glue and Methods for its Preparation and Use" teaches in column 3 last paragraph bridging to column 4, separating the fibrinogen from thrombin, adding calcium chloride, filtering to produce thrombin. The thrombin can be applied to a treatment site in a patient with a syringe. Autologous donations are discussed in column 3.

The claims differ from the above references in that they recite ethanol is an additive to enrich prothrombin in a plasma fraction. On page 23 of the present specification, various ethanol concentrations and the resultant thrombin lifespan are discussed. However, no amounts or concentrations of ethanol are claimed nor any thrombin lifespans.

As the claims are written, it is difficult for the examiner to determine any point of novelty. See Page 23, "Solutions

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

such as saline, dilute CaCl2 or even water added to the thrombin can alter both the clotting time and life span of the thrombin."

It would have been obvious to one of ordinary skill in the art at the time the invention was made to employ any desired physiologically acceptable diluent to dilute the thrombin product in view of the above references. Further, it is not seen that ethanol enriches prothrombin in a plasma fraction. To select any well known diluent for its known function would have been obvious in view of the above references. Altering the clotting time by diluting the thrombin fraction or by using a desired quantity at a given concentration is taught by the references.

---------- NEW CITATIONS ----------

NONE

clotting and adhesive proteins are topically applied to the patient's surgical wound sites.

It has long been understood, however, that the safest condition for a surgical patient would result from a two component biological sealant preparation in which the thrombin component would be harvested from the same donor in which the clotting and adhesive protein component was harvested - forming a fully autologous biological sealant or glue.

The concept of utilizing thrombin and/or fibrinogen sourced from the patient in a medical procedure performed on that patient is not novel and was first described by Andrianova in 1974. Some twenty years later, Cederholm-Williams PCT Patent (WO94/00566 - 6 January 1994 and its related U.S. Patent No. 5,795,780) describes an improved fibrin glue in which the thrombin component, which required thirteen steps, including centrifugation, and separation of intermediate precipitates and adjusting the ionic strength of the blood and pH of the plasma to prepare, would be combined with a fibrinogen component also sourced from the plasma of the same donor. However, these many preparation steps are so time consuming they become impractical for use in the perioperative theater where processing times should be less than one hour. The present invention, *inter alia*, is distinguished in that it is undiluted by pH adjustment.

Three years later, in 1997, Hirsh, et al. (U.S. Patent No. 5,643,192 and its related WO96/31245) follows Cederholm-Williams by teaching another method of preparing fibrin glue in which both the fibrinogen and thrombin components of a fibrin glue are sourced from the same donor's plasma. The Hirsh patent describes a method of preparing thrombin in which most of the fibrinogen in the plasma is first precipitated and removed to prepare a supernatant and then clotting the residual fibrinogen in the supernatant which is different and simpler than the method taught by Cederholm-Williams, but does not result in a commercially useful thrombin in that (see figure 1 of Hirsh, et al.) the thrombin provides clotting speeds of five seconds or less for only 4 minutes, and less than 10 seconds for only 47 minutes. The present invention, *inter alia*, is distinguished in that the plasma is unprocessed as for example by not precipitating out fibrinogen.

These clotting speeds are unsuitable to the needs of surgeons who could not plan their entire surgeries around the limitations of the Hirsh, et al. fibrin glue.

Surgeons predominately require a fast acting clotting time (< 5 seconds) for hemostasis and tissue sealing or adhesion. Slow clotting biological glues (> 5 seconds) will often be transported away from the wound site by oozing and bleeding before they can perform their function. A surgeon utilizing the Hirsh fibrin glue would be required to arrange his surgery so that the hemostasis and tissue sealing intended for treatment with the Hirsh fibrin glue would occur within the 4 minute window where the clotting time was less than 5 seconds, making the Hirsh invention totally impractical for most surgeries which predominantly require rapid hemostasis and tissue adhesion throughout the surgery, the time span of which could extend to six hours.

The following prior art reflects the state of the art of which applicant is aware and is included herewith to discharge applicant's acknowledged duty to disclose relevant prior art. It is stipulated, however, that none of these references teach singly nor render obvious when considered in any conceivable combination the nexus of the instant invention as disclosed in greater detail hereinafter and as particularly claimed.

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The other prior art listed above, not all of which are specifically discussed catalog the prior art of which the applicant is aware. These undiscussed references diverge even more starkly from the instant invention specifically distinguished below.

Disclosure of Invention

The instant invention addresses the long felt need for a simple, practical, fast method of preparing stable human thrombin from a donor's blood, which will provide fast clots (< 5 seconds) throughout a lengthy surgery (*e.g.* six hours) to combine with the clotting and adhesive proteins harvested and concentrated from the same unit of blood to form a biological sealant with no patient exposure to microbial or possible CJD or NVCJD contaminations. Previous works in the field (Hirsch, et al.) exemplified a thrombin with minimal stability in that the thrombin achieved rapid clotting of fibrinogen (*i.e.*, less than 5 seconds) during only a very narrow four to five minute time period, or required so many steps and elapsed time it would not be suitable for perioperative preparation, both totally impractical for the broad range of surgeries.

The present invention provides a stable thrombin enzyme which can cause precise, repeatable fast or slow polymerization of clotting and adhesive proteins over a duration of up to six hours - throughout even a long surgery. Further, the use of clotting and adhesive proteins and thrombin all sourced from a single donor will eliminate various disease risks posed from the use of commercial fibrin glues where the fibrinogen is sourced from plasma pooled from thousands of donors and the thrombin is either sourced from a similar pool of human plasma or of bovine origin. The speed and simplicity of the production of stable thrombin by use of this invention allows it to be prepared just prior to or during operative procedures and

it will provide fast clotting throughout even the longest surgeries. The thrombin produced by this invention can be diluted in saline, water and a dilute $CaCl_2$ solution (*e.g.* 125 mM $CaCl_2$) to provide precise, slower clotting times thereby allowing any preferred time from less than five seconds to longer than 2 minutes.

The procedure of the invention is preferably comprised of three steps, the first two of which should preferably occur at the same time:

1. Preparing a fraction enriched in prothrombin by use of an alcohol, preferably Ethanol to substantially enhance the concentration of prothrombin and at the same time remove or denature naturally occurring ingredients within plasma, such as Fibrinogen and Antithrombin III which can bind to, block, interfere with or inhibit prothrombin or its subsequent activation to long-term functional thrombin.

2. Adding calcium ions to the enriched prothrombin solution and briefly agitating the solution to convert the pro-thrombin to stable, long term thrombin.

3. Expressing the thrombin solution through a filter to remove particulate matter which would prevent spraying the thrombin through a small orifice or expressing the thrombin through a thin tube onto a wound site.

Industrial Applicability

The industrial applicability of this invention shall be demonstrated through discussion of the following objects of the invention.

Accordingly, it is a primary object of the present invention to provide a new and novel apparatus and method to derive fast acting, stable autologous thrombin from the donor's plasma.

It is a further object of the present invention to provide thrombin as characterized above which has a shelf life longer than most associated surgical procedures.

It is a further object of the present invention to provide thrombin as characterized above in which the clotting time can be predictably lengthened at will through dilution with saline.

It is a further object of the present invention to provide thrombin as characterized above which has simple preparatory procedures.

It is a further object of the present invention to provide a method for producing thrombin as characterized above which has a process time in as little as thirty minutes, up to seventy-five minutes.

It is a further object of the present invention to provide thrombin which can be sprayed through small orifices or expressed through thin tubes.

Viewed from a first vantage point it is the object of the present invention to provide a novel and practical method for producing stable human thrombin from a prothrombin fraction which has been substantially enriched by ethanol fractionation to increase the prothrombin concentration and at the same time remove contaminating proteins. The addition of calcium chloride ($CaCl_2$) to the enriched prothrombin converts prothrombin to thrombin. From the same sole donor plasma, clotting and adhesive proteins are simultaneously obtained by other means to comprise the second component necessary for the autologous biological sealant.

Viewed from a second vantage point, it is an object of the present invention to provide a method for generating autologous thrombin from a patient, the steps including: obtaining a blood product from the patient; sequestering plasma from the product; enriching the prothrombin in a plasma fraction; converting the prothrombin to thrombin, and filtering particulate from the thrombin.

Viewed from a third vantage point, it is an object of the present invention to provide a method for producing autologous thrombin which is stable for more than fifteen minutes, the steps including: sequestering pro-thrombin from plasma and converting the pro-thrombin to thrombin.

Viewed from a fourth vantage point, it is an object of the present invention to provide an autologous thrombin which provides fast clotting in less than five seconds for more than fifteen minutes.

Viewed from a fifth vantage point, it is an object of the present invention to provide a composition for extracting thrombin from plasma consisting essentially of: Plasma; Ethanol (ETOH); $CaCl_2$.

Viewed from a sixth vantage point, it is an object of the present invention to provide a method for preparing thrombin comprising: obtaining plasma; adding ETOH and $CaCl_2$ to the plasma, forming a composition: agitating the composition;

incubating the composition in a static or rocking mode; filtering the composition of particulate, thereby passing the thrombin through the filter.

Viewed from a seventh vantage point, it is an object of the present invention to provide a device for preparing thrombin from plasma, comprising: a reaction chamber having a solution of $CaCl_2$ and ETOH therein; means for admitting plasma into the reaction chamber; thrombin receiving syringe coupled to the reaction chamber to receive the thrombin; and a filter located between the reaction chamber and the thrombin receiving syringe.

Viewed from an eighth vantage point, it is an object of the present invention to provide an autologous biological glue processing device, comprising, in combination: a thrombin processing means, a clotting and adhesive proteins processing means operatively coupled to the thrombin processing means, means for receiving plasma via the operative coupling for subsequent conversion of the plasma to, respectively thrombin and clotting and adhesive proteins.

The present invention provides a method and apparatus that produces thrombin which is sufficiently stable that it can provide less-than-5-second clots for up to six hours, substantially more stable than demonstrated in all prior art. Further, the clot time can be modified at will through dilution with saline.

The present invention further provides an efficient method of preparation. Improved cryoprecipitation of clotting and adhesive proteins through the CryoSeal™ invention requires less than one hour. In this same time frame, the autologous human thrombin component can be manufactured with minimal materials and methods from the same source plasma. Both of the biological components of the biological glue are easily combined in a surgical setting, administered to the very same donor patient, and the resultant clotting provides hemostasis or tissue adhesion at the wound site.

The present invention additionally provides a method for sterile production of both components of the biological glue. The improved sterile manufacturing described herein provides a final product that is essentially free of contamination by non autologous microbes.

These and other objects will be made manifest when considering the following detailed specification when taken in conjunction with the appended drawing figures.

Brief Description Of Drawings

Figures 1A and 1B are perspective views of apparatuses for sequestering prothrombin from plasma, processing the prothrombin into thrombin and taking the plasma not relegated towards the prothrombin and extracting clotting and adhesive proteins therefrom.

Figures 2A and 2B are plan views of the thrombin processing sets removed from the processing sets that extracts clotting and adhesive proteins.

Figures 3A and 3B are perspective views of the interior of the thrombin processing cases with the thrombin syringe shown in figures 2A and 2B removed therefrom.

Figures 4A and 4B are perspective views of the thrombin cases upper halves.

Figures 5A and 5B are perspective views of the thrombin cases lower halves.

Figures 6A and 6B are exploded parts views of the reaction chamber 26 shown in figures 3A and 3B along with the valving structure at opposed ends thereof.

Figures 7A and 7B are sectional views of the reaction chambers and valving structures depicted in figures 6A and 6B.

Figures 8A and 8B are detail of construction of that which is shown in figures 7A and 7B.

Figures 9A and 9B are exploded parts view of filter alternatives used in figures 3A and 3B.

Figure 10 is a perspective view of that which is shown in figure 9.

Figure 11 graphs clot time versus lifespan of thrombin fractionated at different ETOH concentrations.

Figure 12 graphs clot time versus lifespan of thrombin fractionated at different ETOH concentrations at different $CaCl_2$ concentrations.

Figure 13 graphs clot time versus lifespan of thrombin showing reagent volume sensitivity when the thrombin is stored on ice.

Figure 14 graphs clot time versus lifespan of thrombin showing reagent volume sensitivity when the thrombin is stored at room temperature.

Figure 15 graphs clot time versus lifespan of thrombin showing plasma volume sensitivity when the thrombin is stored on ice.

Figure 16 graphs clot time versus lifespan of thrombin showing plasma volume sensitivity when the thrombin is stored at room temperature.

Best Mode(s) for Carrying Out the Invention

Referring to the drawings, wherein like elements denote like parts throughout, reference numeral 10 is directed to the processing set according to the present invention and shown in figures 1A and 1B.

In its essence, the processing set 10 includes a fluid receiving system 20 which communicates with both a thrombin processing unit 40 and a clotting and adhesive proteins processing unit 60.

More particularly, viewing both figures 1A and 1B, the fluid receiving system 20 includes an inlet 2 communicating with tubing 4 through which plasma will enter the processing units 40, 60. The conduit 4 has plural positions for stop valves 6 which can occlude the tubing 4 preventing fluids through passage. The tubing 4 communicates through a T fitting 8 to divide plasma into two branches, a first branch 12 which leads to the thrombin processing unit 40 and a second branch 14 leading to the clotting and adhesive proteins processing unit 60. The first valve branch 12 also includes a stop valve 6.

Viewing figure 1B, prior to the introduction of plasma through the first branch 12 thrombin processing unit 40, reagent from preloaded syringe 95 is injected pushing plunger mechanism 94 in the direction of A', into receiving system 20 through sterile barrier filter 92. The reagent passes through one way valve 91; Y connector 90, that merge coupling 18 and valve 91, through branch tubing 93; and finally into the interior of casing 22. Referring to figure 3B and 7B, a valve 24 initially directs the reagent to a reaction chamber 26.

Since it is preferred that the blood product admitted to the inlet 2 be plasma, the whole blood is first processed either by filtering, centrifugation, or another means of settling to remove the heavier red blood cells from the blood products, leaving plasma therebeyond for use in the figure 1 device. Although this system

can be dimensioned for any size batch, the plasma required for the thrombin processing unit will typically be 9-10 ml so that the final volume of concentrated thrombin matches a typical yield of cryoprecipitated clotting and adhesive proteins from the clotting and adhesive proteins processing unit 60.

In the embodiments shown in figures 1A and 1B, sealed bags 16 and 78 overlie both the thrombin dispensing syringe 42 (and a lead in of conduit 64) and cryoprecipitate storage tube 76 to provide sterility until both storage containers are introduced into a sterile surgical field (e.g., operatory). Prior to that, the thrombin processing unit 40 operates as shown and described with reference to figures 2A through 10. Viewing figure 1B, after reagent is added, plasma enters the first branch 12 and passes beyond a coupling 18, through tubing branch 93, and into an interior of the casing 22.

Referring back to figure 1A, the thrombin processing unit 40 operates as shown and described with reference to figures 2A, 3A, 4A, 5A, 6A, 7A, 8A, 9A and 10. As mentioned, fluid enters the first branch 12 and (figure 1A) passes beyond a coupling 18 and into an interior of a casing 22. Coupling 18 is preferably frictionally and/or adhesively attached to the first branch 12 yet the thrombin processing unit 40 can still be removed (e.g. figure 2A) from the processing set 10 (e.g., by merely detaching or severing branch 12 followed perhaps with heat sealing) after receiving the plasma as shown in figure 2. If adhesive is used, it is a sterile grade for use in an operatory.

Referring to figure 3A, a valve 24 initially directs the plasma to a reaction chamber 26 having an interior tube 28a (figure 6A) preferably formed from glass and capable of receiving a volume, for example 15 ml. Glass tube 28a is preferably shorter than and circumscribed by an overlying barrel 32 preferably formed from PVC. A window 31a in the PVC barrel 32 can be used to gauge and/or verify the contents within the glass tube 28a. Gauging may also include gradations 29, indicating a volume on the glass tube. The glass tube 28a of the reaction chamber 26 receives the plasma from the first branch 12 and into its interior for mixing with reagents preloaded in the glass tube 28a and described hereinafter. As shown in figure 7A, the interior of the glass tube is preferably prefilled only partially with beads 25 preferably formed from borosilicate, glass or ceramic to enhance the reaction and agitation.

Referring to figure 3B, a valve 24 initially directs the plasma to a reaction chamber 26 having tube 28b (figure 6B) preferably formed from clear polycarbonate and capable of receiving a volume, for example, 15 ml. Graduated lines 31b on the polycarbonate tube 28b can be used to gauge the contents within the tube 28b. The polycarbonate tube 28b of the reaction chamber 26 receives the plasma from the first branch 12 and into the interior for mixing with reagents previously added into the polycarbonate tube 28b and described hereinafter. As shown in figure 7B, the interior of the tube 28b is preferably prefilled only partially with beads 25 preferably formed from borosilicate or ceramic to enhance the reaction and agitation.

The reaction chamber 26 of the embodiment shown in figures 1A and 3A is formed with first and second end caps 34 detailed in figures 6A, 7A and 8A. Each end cap includes a central outwardly conically tapering spout 36 which communicates with the valve 24 at one end and a further valve 44 at an opposite end. Each spout 36 is isolated from the beads 25 by a screen 23 nested within necked-down portion 48. Valve 24 has three branches as does valve 44, but valve 44 has one branch capped off with a cap 45 thereby defining a two branch valve. One branch of each valve 24, 44 communicates with a respective one spout 36 projecting out from each cap 34. Fluid communication exists between one branch of each valve and its spout into the interior of the glass tube 28a and through flow is controlled by the valves 24, 44. As shown in figure 8A, the cap 34 includes an annular necked-down portion 48a which frictionally and/or adhesively resides within an interior hollow of the PVC barrel 32. In this way, the necked-down portion 48 rests upon ends of the glass tube 28a in sealing engagement therewith, isolating the interior of the reaction chamber from the PVC barrel 32.

For the embodiment forming the reaction chamber 26 of the embodiment shown in figures 1B and 3B mainly out of polycarbonate tube 28 is detailed in figures 6B, 7B and 8B. This reaction chamber 26 is formed with first and second end caps 34 detailed in figure 8B. Each end cap includes a central outwardly conically tapering spout 36 which communicates with the valve 24 at one end and a further valve 44 at an opposite end. Each spout 36 has interior obstructions preventing passage of beads 25 while allowing passage of fluid. Valve 24 has three branches as does valve 44, but valve 44 has one branch capped off with a cap 45 thereby defining a two branch valve. One branch of each valve 24, 44 communicates with a

respective one spout 36 projecting out from each cap 34. Fluid communication exists between one branch of each valve and its spout into the interior of the polycarbonate tube 28b and through flow is controlled by the valves 24, 44. As shown in figure 8B, the cap 34 includes an annular interior recess portion 48b which adhesively resides on the interior surface of the polycarbonate tube 28b.

Preferably, ethanol and calcium chloride are the reagents which have been preloaded into the reaction chamber 26 or within reagent syringe 95. Initially, both valves 24 and 44 are oriented so that reagents will not pass therebeyond to seal the chamber for the embodiment of figure 1A. Viewing figure 1B, initially valve 24 is oriented so plasma will not enter reaction chamber 26, and valve 44 is oriented to allow passageway between the reaction chamber 26 and the draw plunger 56. Referring back to figure 1A, after the plasma has been pumped into processing unit 60, valve 44 is turned to allow access to the draw plunger 56 and valve 24 is oriented to allow access between the passageway 21 and the reaction chamber 26. Slide clip 6 is opened with the thrombin processing unit 40 held vertically with respect to the plan shown in figure 1A, syringe ~~56~~ plunger 58 is moved along the direction of the arrow A to evacuate air from chamber 26. Referring back to figure 1B, the reagent syringe 95 is attached to open end of sterile barrier filter 92. Plunger 94 is depressed to transfer reagent syringe through sterile barrier filter and passageway 93 to reaction chamber 26. Likewise to the figure 1A embodiment, the figure 1B, with the thrombin processing unit 40 held vertically with respect to the plan shown in figure 1B, the syringe plunger 58 is moved along the direction of the arrow A to evacuate air from chamber 26. In both embodiments syringe 56 includes a filter 62 located in the flow path. More specifically, the path 43 between valve 44 and syringe 56 includes a filter 62 located in the flow path. The filter 62 provides an aesceptic microbial barrier so that, upon subsequent delivery of the thrombin to the dispensing syringe 42 (figure 1), there is no contamination from around the seal 57 of plunger 58 delivered to syringe 42. Plasma will subsequently enter chamber 26 from conduit 4 to replace air. Valve 24 is oriented to address filter 66. The reagents and plasma are briefly agitated assisted by beads 25 (and allowed to incubate for about 40 to 70 minutes). After incubation, thrombin processing unit 40 is agitated to loosen and break up gel formation. For the embodiment of figure 1B, the thrombin processing unit 40 is then returned to a motionless horizontal position for no less

than 10 minutes. Afterwards the thrombin processing unit 40 is again agitated to loosen and break up gel formation. For both embodiments, the plunger of syringe 56 is pushed in the direction opposite arrow A to move thrombin from chamber 26 through filter 66 into syringe 42. Delivery of thrombin to syringe 42 can be enhanced by retracting plunger 43 of syringe 42, defining a push pull system. Filter 66 removes particulate matter from the thrombin, including gel.

By allowing the thrombin contained in the reaction chamber 26 to reside therein after agitation for no less than 10 minutes enhances the effectiveness of the filter 66 in removing particulate matter for subsequent utilization. The time span for conversion and activation allows enough particulate matter to be removed by the filter to optimize the use of the thrombin later in a narrow orificed dispenser, such as a sprayer, or expression through a thin tube.

Figures 9A, 9B and 10 reveal alternative embodiments of filter 66 which includes an outer cylindrical wall 65 with end caps 34 each having a cylindrical spout 37 circumscribed by an annular recess 39. The alternative embodiment shown in figure 9A shows the centrally disposed cylindrical filter element 67a is preferably formed from polyurethane foam. While as shown in figure 9B the centrally disposed cylindrical filter element 67b is preferably formed from rolled polyester. Also shown in figure 9B, are circular filters 68 preferably formed from glass fiber or polyester. In each alternative embodiment, filter 67a or 67b filters by weight, size and protein binding.

Referring back to figures 1A and 1B, attention is now directed to the clotting and adhesive protein processing unit 60. All of the plasma not diverted to the thrombin processing unit 40 is admitted to an interior chamber 72 of the clotting and adhesive protein processing unit 60. The clotting and adhesive protein processing unit 60 is manipulated by heat exchange and rotation so that all clotting and adhesive proteins extracted from the plasma will sediment at a nose 74 of the chamber 72 for subsequent extraction by means of a clotting and adhesive protein collection tube or dispensing syringe 76 contained in a sterile pouch 78. Chamber 72 is protected during this process by a filter vent 82 preventing contamination. Once the thrombin has been loaded into the dispensing syringe 42, and the clotting and adhesive proteins have been loaded into the clotting and adhesive collection tube or dispensing syringe 76, the two storage containers 42, 76 can be decoupled from the

processing set 10 (e.g. sterile disconnect device), and passed near the sterile, surgical arena. The overwrap bags are subsequently opened, and the storage containers 42, 76 are decoupled and transferred into the surgical area where the contents are dispensed into individual sterile 3cc plastic syringes which are subsequently loaded into the fibrin glue applicator for spraying or line and dot application. Mixing the thrombin with the clotting and adhesive proteins forms the biological glue.

Both dispensing syringes 42 and 76 are stored at room temperature, or preferably stored at their optimal conditions: cryoprecipitate 76 being stored at room temperature and thrombin 42, stored in an ice bath at 1°C to 5°C. Please see figures 13 through 16.

Assume 9-10 ml of room temperature plasma is introduced into the reaction chamber 26. Other plasma volumes have utility. Please see figures 15 and 16. Add 1.0 ml of 75 mM calcium chloride ($CaCl_2$) and 2.0 ml of ethanol (ETOH) (i.e., ethanol taken from a 100% "stock" bottle and added to comprise 18.9% volume/unit volume or 15.02% ethanol weight/unit volume). Other ratios of reagent volume, comprising of ethanol (ETOH) (i.e., ethanol taken from a 100% "stock" bottle and a stock solution of 75 mM calcium chloride ($CaCl_2$)), to plasma volume have utility phase. Please see figures 13 and 14. The thrombin life span is shown to have been at least 300 minutes while its clotting time is at 2.98 seconds. An ethanol final concentration range between 8.0% and 20.0% (volume/unit volume), however, still has utility. Please see figure 11.

When the ethanol is at a final concentration of 18.9% volume/unit volume (as above) and the calcium chloride final concentration is 5.7 mM (1 ml taken from a 75 mM stock solution of calcium chloride), the thrombin lifespan also extends to at least 360 minutes while maintaining a clot time of 5.98 seconds when thrombin is stored at room temperature. Storing thrombin in optimal 1°C to 5°C ice bath typically maintains lot times of 2 to 3 seconds at 360 minutes. Calcium chloride stock solution concentrations ranging between 50 mM and 250 mM, however, have utility. Please see figure 12. The final concentrations range from 4.5mM to 23 mM.

Solutions such as saline, dilute $CaCl_2$ (e.g. 40mM to 125 mM $CaCl_2$) or even sterile water added to the thrombin can alter both the clotting time and life span of the thrombin. Assume an ethanol final concentration of 18.9% and a final calcium

chloride concentration of 5.7 mM was used in the reaction chamber 26. When the thrombin has been diluted 1 to 1.5 with water, the clot time has been extended to just less than 30 seconds, and has a life span of up to 150 minutes.

Moreover, having thus described the invention, it should be apparent that numerous structural modifications and adaptations may be resorted to without departing from the scope and fair meaning of the instant invention as set forth hereinabove and as described hereinbelow by the claims.

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Claims

We Claim:

Claim 1 - A method for generating autologous thrombin from a patient, the steps including:

obtaining a blood product from the patient;

sequestering unadulterated plasma from the product;

enriching the prothrombin in an unadulterated plasma fraction;

converting the prothrombin to thrombin, and

filtering particulate from the thrombin.

Claim 2 - The method of claim 1 further including the step of altering the clotting time.

Claim 3 - The method of claim 2 including adding ethanol to enrich the prothrombin in a plasma fraction.

Claim 4 - The method of claim 3 wherein the converting step includes adding a source of calcium ions.

Claim 5 - The method of claim 4 including centrifuging the blood product for obtaining unadulterated plasma.

Claim 6 - The method of claim 2 wherein a predictable clotting time extension occurs through diluting the thrombin with any of the group consisting of saline, $CaCl_2$ solution and sterile water.

Claim 7 - The method of claim 6 including filtering the plasma by weight, size and protein binding.

Claim 8 - A method for producing fast clotting autologous thrombin which is stable for more than fifteen minutes, the steps including:

sequestering prothrombin from unadulterated plasma and converting the prothrombin to thrombin.

Claim 9 - Autologous thrombin which provides fast clotting in less than five seconds and is stable for more than fifteen minutes.

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Claim 10 - A composition for extracting thrombin from plasma consisting essentially of:

unadulterated Plasma;

Ethanol (ETOH);

$CaCl_2$.

Claim 11 - The composition of claim 10 wherein ETOH is present at 18.9% and $CaCl_2$ is present at 23.0 mM both by volume in final concentration.

Claim 12 - The composition of claim 10 wherein ETOH is present at 18.9% and $CaCl_2$ is present at 5.7 mM both by volume in final concentration.

Claim 13 - The composition of claim 10 wherein ETOH is present at a range between 8% and 20% and $CaCl_2$ is present at a range between 4.5 mM and 23.0 mM both by volume in final concentration.

Claim 14 - A method for preparing thrombin comprising:

obtaining unadulterated plasma;

adding ETOH and $CaCl_2$ to the unadulterated plasma, forming a composition:

agitating the composition;

filtering the composition of particulate, thereby passing the thrombin through the filter.

Claim 15 - The method of claim 14 whereby subsequent to agitating the composition, incubating the composition for an amount of time greater than or equal to ten minutes.

Claim 16 - The method of claim 15 whereby prior to filtering the composition, re-agitating the composition.

Claim 17 - A device for preparing thrombin from plasma, comprising:

a reaction chamber having a solution of $CaCl_2$ and ETOH therein;

means for admitting unadulterated plasma into said reaction chamber;

a thrombin receiving syringe coupled to said reaction chamber to receive the thrombin; and

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a filter located between said reaction chamber and said thrombin receiving syringe.

Claim 18 - A single donor biological glue processing device, comprising, in combination:

a thrombin processing means,

a clotting and adhesive proteins processing means operatively coupled to said thrombin processing means,

means for receiving plasma via said operative coupling for subsequent conversion of the plasma to, respectively thrombin in said thrombin processing means and clotting and adhesive proteins in said clotting and adhesive proteins processing means.

Claim 19 - A device for preparing thrombin from plasma, comprising:

a reaction chamber having ceramic beads or borosilicate glass therein;

means for admitting a reagent into said reaction chamber;

means for admitting plasma into said reaction chamber;

a thrombin receiving syringe coupled to said reaction chamber to receive the thrombin; and

a filter located between said reaction chamber and said thrombin receiving syringe.

Claim 20 - The device of claim 19 wherein the reagent includes $CaCl_2$ and ETOH solution.

Claim 21 - The method of claim 1 further including the step of contacting the plasma with glass beads.

Claim 22 - A composition for extracting thrombin from plasma consisting essentially of:

plasma;

ethanol (ETOH);

$CaCl_2$; and

glass beads.

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Claim 23 - The composition of claim 22 wherein ETOH is present at 18.9% and $CaCl_2$ is present at 23.0 mM both by volume in final concentration.

Claim 24 - The composition of claim 22 wherein ETOH is present at 18.9% and $CaCl_2$ is present at 5.7 mM both by volume in final concentration.

Claim 25 - The composition of claim 22 wherein ETOH is present at a range between 8% and 20% and $CaCl_2$ is present at a range between 4.5 mM and 23.0 mM both by volume in final concentration.

Claim 26 - An apparatus to prepare thrombin from plasma consisting of:

a reacting chamber to accept $CaCl_2$ and ethanol, and means for delivery of plasma into said reacting chamber;

a syringe connected to said reacting chamber to receive thrombin from said reacting chamber;

and a filter between said reacting chamber and syringe which is to receive thrombin.

Claim 27 - The apparatus of claim 26 further including glass beads in said reacting chamber.

Claim 28 - A method for generating and then dispensing thrombin, the steps including:

taking whole blood from one person,

sequestering prothrombin from the whole blood,

converting the prothrombin to thrombin,

loading the thrombin into a syringe,

and then applying the thrombin onto an area to stem blood flow.

Claim 29 - The method of claim 28 including loading clotting proteins into another syringe and dispensing the clotting proteins concurrently with the thrombin.

Claim 30 - A method for generating thrombin from one person, the steps including:

sequestering prothrombin from plasma taken from the person,

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converting the prothrombin to thrombin and removing particulate material therefrom.

Claim 31 - The method of claim 30 further including diluting the thrombin in order to alter clotting time of the thrombin.

Claim 32 - The method of claim 31 including adding a source of calcium ions to alter the clotting time.

Claim 33 - The method of claim 32 including adding $CaCl_2$ to alter the clotting time.

Claim 34 - The method of claim 31 including adding saline to alter the clotting time.

Claim 35 - The method of claim 31 including adding sterile water to alter the clotting time.

Claim 36 - The method of claim 2 wherein the step of altering the clotting time includes adding a source of calcium ions.

Claim 37 - The method of claim 2 wherein the step of altering the clotting time includes adding $CaCl_2$.

Claim 38 - The method of claim 2 wherein the step of altering the clotting time includes adding saline.

Claim 39 - The method of claim 2 wherein the step of altering the clotting time includes adding sterile water.

Claim 40 - A method for generating thrombin from one person, the steps including:

taking whole blood from the one person,

obtaining plasma from the whole blood,

converting prothrombin in the plasma to thrombin and

sequestering the thrombin from the plasma.

Claim 41 - The method of claim 40 including altering the clotting time of the thrombin to clot in less than five seconds.

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Claim 42 - The method of claim 41 wherein the step of altering the clotting time includes adding a source of calcium ions.

Claim 43 - The method of claim 42 wherein the step of altering the clotting time includes adding $CaCl_2$.

Claim 44 - The method of claim 41 wherein the step of altering the clotting time includes adding saline.

Claim 45 - The method of claim 41 wherein the step of altering the clotting time includes adding sterile water.

Claim 46 - The method of claim 40 including making the thrombin stable for more than fifteen minutes.

Claim 47 - The method of claim 46 including adding a source of calcium ions to alter the clotting time.

Claim 48 - The method of claim 47 including adding $CaCl_2$ to alter the slotting time.

Claim 49 - The method of claim 46 including adding saline to alter the clotting time.

Claim 50 - The method of claim 46 including adding sterile water to alter the clotting time.

Claim 51 - The method of claim 1, the steps including adding the reagents ETOH and $CaCl_2$ to the plasma followed by agitation to form the thrombin.

Claim 52 - The method of claim 51 further including:

taking whole blood from one person,

sequestering prothrombin from the whole blood,

converting the prothrombin to thrombin,

loading the thrombin into a syringe,

and then applying the thrombin onto an area to stem blood flow.

Claim 53 - The device of claim 18 including a thrombin syringe coupled to said thrombin processing means to receive thrombin therefrom, said thrombin syringe initially ensconced in a bag, and

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a clotting and adhesive protein syringe coupled to said clotting and adhesive protein processing means to receive clotting and adhesive proteins therefrom, said clotting and adhesive protein syringe initially ensconced in a bag.

clotting and adhesive proteins are topically applied to the patient's surgical wound sites.

It has long been understood, however, that the safest condition for a surgical patient would result from a two component biological sealant preparation in which the thrombin component would be harvested from the same donor in which the clotting and adhesive protein component was harvested - forming a fully autologous biological sealant or glue.

The concept of utilizing thrombin and/or fibrinogen sourced from the patient in a medical procedure performed on that patient is not novel and was first described by Andrianova in 1974. Some twenty years later, Cederholm-Williams PCT Patent (WO94/00566 - 6 January 1994) describes an improved fibrin glue in which the thrombin component, which required thirteen steps, including centrifugation, and separation of intermediate precipitates and adjusting the ionic strength of the blood and pH of the plasma to prepare, would be combined with a fibrinogen component also sourced from the plasma of the same donor. However, these many preparation steps are so time consuming they become impractical for use in the perioperative theater where processing times should be less than one hour.

Three years later, in 1997, Hirsh, et al. (U.S. Patent No. 5,643,192) follows Cederholm-Williams by teaching another method of preparing fibrin glue in which both the fibrinogen and thrombin components of a fibrin glue are sourced from the same donor's plasma. The Hirsh patent describes a method of preparing thrombin in which most of the fibrinogen in the plasma is first precipitated and removed to prepare a supernatant and then clotting the residual fibrinogen in the supernatant which is different and simpler than the method taught by Cederholm-Williams, but does not result in a commercially useful thrombin in that (see figure 1 of Hirsh, et al.) the thrombin provides clotting speeds of five seconds or less for only 4 minutes, and less than 10 seconds for only 47 minutes.

These clotting speeds are unsuitable to the needs of surgeons who could not plan their entire surgeries around the limitations of the Hirsh, et al. fibrin glue.

Surgeons predominately require a fast acting clotting time (< 5 seconds) for hemostasis and tissue sealing or adhesion. Slow clotting biological glues (> 5 seconds) will often be transported away from the wound site by oozing and bleeding

before they can perform their function. A surgeon utilizing the Hirsh fibrin glue would be required to arrange his surgery so that the hemostasis and tissue sealing intended for treatment with the Hirsh fibrin glue would occur within the 4 minute window where the clotting time was less than 5 seconds, making the Hirsh invention totally impractical for most surgeries which predominantly require rapid hemostasis and tissue adhesion throughout the surgery, the time span of which could extend to six hours.

The following prior art reflects the state of the art of which applicant is aware and is included herewith to discharge applicant's acknowledged duty to disclose relevant prior art. It is stipulated, however, that none of these references teach singly nor render obvious when considered in any conceivable combination the nexus of the instant invention as disclosed in greater detail hereinafter and as particularly claimed.

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Disclosure of Invention

The instant invention addresses the long felt need for a simple, practical, fast method of preparing stable human thrombin from a donor's blood, which will provide fast clots (< 5 seconds) throughout a lengthy surgery (*e.g.* six hours) to combine with the clotting and adhesive proteins harvested and concentrated from the same unit of blood to form a biological sealant with no patient exposure to microbial or possible CJD or NVCJD contaminations. Previous works in the field (Hirsch, et al.) exemplified a thrombin with minimal stability in that the thrombin achieved rapid clotting of fibrinogen (*i.e.*, less than 5 seconds) during only a very narrow four to five minute time period, or required so many steps and elapsed time it would not be suitable for perioperative preparation, both totally impractical for the broad range of surgeries.

The present invention provides a stable thrombin enzyme which can cause precise, repeatable fast or slow polymerization of clotting and adhesive proteins over a duration of up to six hours - throughout even a long surgery. Further, the use of clotting and adhesive proteins and thrombin all sourced from a single donor will eliminate various disease risks posed from the use of commercial fibrin glues where the fibrinogen is sourced from plasma pooled from thousands of donors and the thrombin is either sourced from a similar pool of human plasma or of bovine origin. The speed and simplicity of the production of stable thrombin by use of this invention allows it to be prepared just prior to or during operative procedures and it will provide fast clotting throughout even the longest surgeries. The thrombin produced by this invention can be diluted in saline, water and a dilute $CaCL_2$

solution (*e.g.* 125 mM $CaCL_2$) to provide precise, slower clotting times thereby allowing any preferred time from less than five seconds to longer than 2 minutes.

The procedure of the invention is preferably comprised of three steps, the first two of which should preferably occur at the same time:

1. Preparing a fraction enriched in prothrombin by use of Ethanol to substantially enhance the concentration of prothrombin and at the same time remove or denature naturally occurring ingredients within plasma, such as Fibrinogen and Antithrombin III which can bind to, block, interfere with or inhibit prothrombin or its subsequent activation to long-term functional thrombin.

2. Adding calcium ions to the enriched prothrombin solution and briefly agitating the solution to convert the pro-thrombin to stable, long term thrombin.

3. Expressing the thrombin solution through a filter to remove particulate matter which would prevent spraying the thrombin through a small orifice or expressing the thrombin through a thin tube onto a wound site.

Industrial Applicability

The industrial applicability of this invention shall be demonstrated through discussion of the following objects of the invention.

Accordingly, it is a primary object of the present invention to provide a new and novel apparatus and method to derive fast acting, stable autologous thrombin from the donor's plasma.

It is a further object of the present invention to provide thrombin as characterized above which has a shelf life longer than most associated surgical procedures.

It is a further object of the present invention to provide thrombin as characterized above in which the clotting time can be predictably lengthened at will through dilution with saline.

It is a further object of the present invention to provide thrombin as characterized above which has simple preparatory procedures.

It is a further object of the present invention to provide a method for producing thrombin as characterized above which has a process time in as little as thirty minutes, up to seventy-five minutes.

It is a further object of the present invention to provide thrombin which can be sprayed through small orifices or expressed through thin tubes.

Viewed from a first vantage point it is the object of the present invention to provide a novel and practical method for producing stable human thrombin from a prothrombin fraction which has been substantially enriched by ethanol fractionation to increase the prothrombin concentration and at the same time remove contaminating proteins. The addition of calcium chloride ($CaCl_2$) to the enriched prothrombin converts prothrombin to thrombin. From the same sole donor plasma, clotting and adhesive proteins are simultaneously obtained by other means to comprise the second component necessary for the autologous biological sealant.

Viewed from a second vantage point, it is an object of the present invention to provide a method for generating autologous thrombin from a patient, the steps including: obtaining a blood product from the patient; sequestering plasma from the product; enriching the prothrombin in a plasma fraction; converting the prothrombin to thrombin, and filtering particulate from the thrombin.

Viewed from a third vantage point, it is an object of the present invention to provide a method for producing autologous thrombin which is stable for more than fifteen minutes, the steps including: sequestering pro-thrombin from plasma and converting the pro-thrombin to thrombin.

Viewed from a fourth vantage point, it is an object of the present invention to provide an autologous thrombin which provides fast clotting in less than five seconds for more than fifteen minutes.

Viewed from a fifth vantage point, it is an object of the present invention to provide a composition for extracting thrombin from plasma consisting essentially of: Plasma; Ethanol (ETOH); $CaCL_2$.

Viewed from a sixth vantage point, it is an object of the present invention to provide a method for preparing thrombin comprising: obtaining plasma; adding ETOH and $CaCL_2$ to the plasma, forming a composition: agitating the composition; incubating the composition in a static or rocking mode; filtering the composition of particulate, thereby passing the thrombin through the filter.

Viewed from a seventh vantage point, it is an object of the present invention to provide a device for preparing thrombin from plasma, comprising: a reaction

chamber having a solution of $CaCL_2$ and ETOH therein; means for admitting plasma into the reaction chamber; thrombin receiving syringe coupled to the reaction chamber to receive the thrombin; and a filter located between the reaction chamber and the thrombin receiving syringe.

Viewed from an eighth vantage point, it is an object of the present invention to provide an autologous biological glue processing device, comprising, in combination: a thrombin processing means, a clotting and adhesive proteins processing means operatively coupled to the thrombin processing means, means for receiving plasma via the operative coupling for subsequent conversion of the plasma to, respectively thrombin and clotting and adhesive proteins.

The present invention provides a method and apparatus that produces thrombin which is sufficiently stable that it can provide less-than-5-second clots for up to six hours, substantially more stable than demonstrated in all prior art. Further, the clot time can be modified at will through dilution with saline.

The present invention further provides an efficient method of preparation. Improved cryoprecipitation of clotting and adhesive proteins through the CryoSeal™ invention requires less than one hour. In this same time frame, the autologous human thrombin component can be manufactured with minimal materials and methods from the same source plasma. Both of the biological components of the biological glue are easily combined in a surgical setting, administered to the very same donor patient, and the resultant clotting provides hemostasis or tissue adhesion at the wound site.

The present invention additionally provides a method for sterile production of both components of the biological glue. The improved sterile manufacturing described herein provides a final product that is essentially free of contamination by non autologous microbes.

These and other objects will be made manifest when considering the following detailed specification when taken in conjunction with the appended drawing figures.

Brief Description Of Drawings

Figures 1A and 1B are perspective views of apparatuses for sequestering prothrombin from plasma, processing the prothrombin into thrombin and taking

the plasma not relegated towards the prothrombin and extracting clotting and adhesive proteins therefrom.

Figures 2A and 2B are plan views of the thrombin processing sets removed from the processing sets that extracts clotting and adhesive proteins.

Figures 3A and 3B are perspective views of the interior of the thrombin processing cases with the thrombin syringe shown in figures 2A and 2B removed therefrom.

Figures 4A and 4B are perspective views of the thrombin cases upper halves.

Figures 5A and 5B are perspective views of the thrombin cases lower halves.

Figures 6A and 6B are exploded parts views of the reaction chamber 26 shown in figures 3A and 3B along with the valving structure at opposed ends thereof.

Figures 7A and 7B are sectional views of the reaction chambers and valving structures depicted in figures 6A and 6B.

Figures 8A and 8B are detail of construction of that which is shown in figures 7A and 7B.

Figures 9A and 9B are exploded parts view of filter alternatives used in figures 3A and 3B.

Figure 10 is a perspective view of that which is shown in figure 9.

Figure 11 graphs clot time versus lifespan of thrombin fractionated at different ETOH concentrations.

Figure 12 graphs clot time versus lifespan of thrombin fractionated at different ETOH concentrations at different $CaCL_2$ concentrations.

Figure 13 graphs clot time versus lifespan of thrombin showing reagent volume sensitivity when the thrombin is stored on ice.

Figure 14 graphs clot time versus lifespan of thrombin showing reagent volume sensitivity when the thrombin is stored at room temperature.

Figure 15 graphs clot time versus lifespan of thrombin showing plasma volume sensitivity when the thrombin is stored on ice.

Figure 16 graphs clot time versus lifespan of thrombin showing plasma volume sensitivity when the thrombin is stored at room temperature.

Best Mode(s) for Carrying Out the Invention

Referring to the drawings, wherein like elements denote like parts throughout, reference numeral 10 is directed to the processing set according to the present invention and shown in figures 1A and 1B.

In its essence, the processing set 10 includes a fluid receiving system 20 which communicates with both a thrombin processing unit 40 and a clotting and adhesive proteins processing unit 60.

More particularly, viewing both figures 1A and 1B, the fluid receiving system 20 includes an inlet 2 communicating with tubing 4 through which plasma will enter the processing units 40, 60. The conduit 4 has plural positions for stop valves 6 which can occlude the tubing 4 preventing fluids through passage. The tubing 4 communicates through a T fitting 8 to divide plasma into two branches, a first branch 12 which leads to the thrombin processing unit 40 and a second branch 14 leading to the clotting and adhesive proteins processing unit 60. The first valve branch 12 also includes a stop valve 6.

Viewing figure 1B, prior to the introduction of plasma through the first branch 12 thrombin processing unit 40, reagent from preloaded syringe 95 is injected pushing plunger mechanism 94 in the direction of A', into receiving system 20 through sterile barrier filter 92. The reagent passes through one way valve 91; Y connector 90, that merge coupling 18 and valve 91, through branch tubing 93; and finally into the interior of casing 22. Referring to figure 3B and 7B, a valve 24 initially directs the reagent to a reaction chamber 26.

Since it is preferred that the blood product admitted to the inlet 2 be plasma, the whole blood is first processed either by filtering, centrifugation, or another means of settling to remove the heavier red blood cells from the blood products, leaving plasma therebeyond for use in the figure 1 device. Although this system can be dimensioned for any size batch, the plasma required for the thrombin processing unit will typically be 9-10 ml so that the final volume of concentrated thrombin matches a typical yield of cryoprecipitated clotting and adhesive proteins from the clotting and adhesive proteins processing unit 60.

In the embodiments shown in figures 1A and 1B, sealed bags 16 and 78 overlie both the thrombin dispensing syringe 42 (and a lead in of conduit 64) and cryoprecipitate storage tube 76 to provide sterility until both storage containers are introduced into a sterile surgical field (e.g., operatory). Prior to that, the thrombin processing unit 40 operates as shown and described with reference to figures 2A through 10. Viewing figure 1B, after reagent is added, plasma enters the first branch 12 and passes beyond a coupling 18, through tubing branch 93, and into an interior of the casing 22.

Referring back to figure 1A, the thrombin processing unit 40 operates as shown and described with reference to figures 2A, 3A, 4A, 5A, 6A, 7A, 8A, 9A and 10. As mentioned, fluid enters the first branch 12 and (figure 1A) passes beyond a coupling 18 and into an interior of a casing 22. Coupling 18 is preferably frictionally and/or adhesively attached to the first branch 12 yet the thrombin processing unit 40 can still be removed (e.g. figure 2A) from the processing set 10 (e.g., by merely detaching or severing branch 12 followed perhaps with heat sealing) after receiving the plasma as shown in figure 2. If adhesive is used, it is a sterile grade for use in an operatory.

Referring to figure 3A, a valve 24 initially directs the plasma to a reaction chamber 26 having an interior tube 28a (figure 6A) preferably formed from glass and capable of receiving a volume, for example 15 ml. Glass tube 28a is preferably shorter than and circumscribed by an overlying barrel 32 preferably formed from PVC. A window 31a in the PVC barrel 32 can be used to gauge and/or verify the contents within the glass tube 28a. Gauging may also include gradations 29, indicating a volume on the glass tube. The glass tube 28a of the reaction chamber 26 receives the plasma from the first branch 12 and into its interior for mixing with reagents preloaded in the glass tube 28a and described hereinafter. As shown in figure 7A, the interior of the glass tube is preferably prefilled only partially with beads 25 preferably formed from borosilicate, glass or ceramic to enhance the reaction and agitation.

Referring to figure 3B, a valve 24 initially directs the plasma to a reaction chamber 26 having tube 28b (figure 6B) preferably formed from clear polycarbonate and capable of receiving a volume, for example, 15 ml. Graduated lines 31b on the polycarbonate tube 28b can be used to gauge the contents within the tube 28b. The

polycarbonate tube 28b of the reaction chamber 26 receives the plasma from the first branch 12 and into the interior for mixing with reagents previously added into the polycarbonate tube 28b and described hereinafter. As shown in figure 7B, the interior of the tube 28b is preferably prefilled only partially with beads 25 preferably formed from borosilicate or ceramic to enhance the reaction and agitation.

The reaction chamber 26 of the embodiment shown in figures 1A and 3A is formed with first and second end caps 34 detailed in figures 6A, 7A and 8A. Each end cap includes a central outwardly conically tapering spout 36 which communicates with the valve 24 at one end and a further valve 44 at an opposite end. Each spout 36 is isolated from the beads 25 by a screen 23 nested within necked-down portion 48. Valve 24 has three branches as does valve 44, but valve 44 has one branch capped off with a cap 45 thereby defining a two branch valve. One branch of each valve 24, 44 communicates with a respective one spout 36 projecting out from each cap 34. Fluid communication exists between one branch of each valve and its spout into the interior of the glass tube 28a and through flow is controlled by the valves 24, 44. As shown in figure 8A, the cap 34 includes an annular necked-down portion 48 which frictionally and/or adhesively resides within an interior hollow of the PVC barrel 32. In this way, the necked-down portion 48 rests upon ends of the glass tube 28a in sealing engagement therewith, isolating the interior of the reaction chamber from the PVC barrel 32.

For the embodiment forming the reaction chamber 26 of the embodiment shown in figures 1B and 3B mainly out of polycarbonate tube 28 is detailed in figures 6B, 7B and 8B. This reaction chamber 26 is formed with first and second end caps 34 detailed in figure 8B. Each end cap includes a central outwardly conically tapering spout 36 which communicates with the valve 24 at one end and a further valve 44 at an opposite end. Each spout 36 has interior obstructions preventing passage of beads 25 while allowing passage of fluid. Valve 24 has three branches as does valve 44, but valve 44 has one branch capped off with a cap 45 thereby defining a two branch valve. One branch of each valve 24, 44 communicates with a respective one spout 36 projecting out from each cap 34. Fluid communication exists between one branch of each valve and its spout into the interior of the polycarbonate tube 28b and through flow is controlled by the valves 24, 44. As

shown in figure 8B, the cap 34 includes an annular interior recess portion 48 which adhesively resides on the interior surface of the polycarbonate tube 28b.

Preferably, ethanol and calcium chloride are the reagents which have been preloaded into the reaction chamber 26 or within reagent syringe 95. Initially, both valves 24 and 44 are oriented so that reagents will not pass therebeyond to seal the chamber for the embodiment of figure 1A. Viewing figure 1B, initially valve 24 is oriented so plasma will not enter reaction chamber 26, and valve 44 is oriented to allow passageway between the reaction chamber 26 and the draw plunger 56. Referring back to figure 1A, after the plasma has been pumped into processing unit 60, valve 44 is turned to allow access to the draw plunger 56 and valve 24 is oriented to allow access between the passageway 21 and the reaction chamber 26. Slide clip 6 is opened with the thrombin processing unit 40 held vertically with respect to the plan shown in figure 1A, syringe ~~56~~ plunger 58 is moved along the direction of the arrow A to evacuate air from chamber 26. Referring back to figure 1B, the reagent syringe 95 is attached to open end of sterile barrier filter 92. Plunger 94 is depressed to transfer reagent syringe through sterile barrier filter and passageway 93 to reaction chamber 26. Likewise to the figure 1A embodiment, the figure 1B, with the thrombin processing unit 40 held vertically with respect to the plan shown in figure 1B, the syringe plunger 58 is moved along the direction of the arrow A to evacuate air from chamber 26. In both embodiments syringe 56 includes a filter 62 located in the flow path. More specifically, the path 43 between valve 44 and syringe 56 includes a filter 62 located in the flow path. The filter 62 provides an aesceptic microbial barrier so that, upon subsequent delivery of the thrombin to the dispensing syringe 42 (figure 1), there is no contamination from around the seal 57 of plunger 58 delivered to syringe 42. Plasma will subsequently enter chamber 26 from conduit 4 to replace air. Valve 24 is oriented to address filter 66. The reagents and plasma are briefly agitated assisted by beads 25 (and allowed to incubate for about 40 to 70 minutes). After incubation, thrombin processing unit 40 is agitated to loosen and break up gel formation. For the embodiment of figure 1B, the thrombin processing unit 40 is then returned to a motionless horizontal position for no less than 10 minutes. Afterwards the thrombin processing unit 40 is again agitated to loosen and break up gel formation. For both embodiments, the plunger of syringe 56 is pushed in the direction opposite arrow A to move thrombin from chamber 26

through filter 66 into syringe 42. Delivery of thrombin to syringe 42 can be enhanced by retracting plunger 43 of syringe 42, defining a push pull system. Filter 66 removes particulate matter from the thrombin, including gel.

By allowing the thrombin contained in the reaction chamber 26 to reside therein after agitation for no less than 10 minutes enhances the effectiveness of the filter 66 in removing particulate matter for subsequent utilization. The time span for conversion and activation allows enough particulate matter to be removed by the filter to optimize the use of the thrombin later in a narrow orificed dispenser, such as a sprayer, or expression through a thin tube.

Figures 9A, 9B and 10 reveal alternative embodiments of filter 66 which includes an outer cylindrical wall 65 with end caps 34 each having a cylindrical spout 37 circumscribed by an annular recess 39. The alternative embodiment shown in figure 9A shows the centrally disposed cylindrical filter element 67a is perferably formed from polyurethane foam. While as shown in figure 9B the centrally disposed cylindrical filter element 67b is preferably formed from rolled polyester. Also shown in figure 9B, are circular filters 68 preferably formed from glass fiber or polyester. In each alternative embodiment, filter 67a or 67b filters by weight, size and protein binding.

Referring back to figures 1A and 1B, attention is now directed to the clotting and adhesive protein processing unit 60. All of the plasma not diverted to the thrombin processing unit 40 is admitted to an interior chamber 72 of the clotting and adhesive protein processing unit 60. The clotting and adhesive protein processing unit 60 is manipulated by heat exchange and rotation so that all clotting and adhesive proteins extracted from the plasma will sediment at a nose 74 of the chamber 72 for subsequent extraction by means of a clotting and adhesive protein collection tube or dispensing syringe 76 contained in a sterile pouch 78. Chamber 72 is protected during this process by a filter vent 82 preventing contamination. Once the thrombin has been loaded into the dispensing syringe 42, and the clotting and adhesive proteins have been loaded into the clotting and adhesive collection tube or dispensing syringe 76, the two storage containers 42, 76 can be decoupled from the processing set 10 (e.g. sterile disconnect device), and passed near the sterile, surgical arena. The overwrap bags are subsequently opened, and the storage containers 42, 76 are decoupled and transferred into the surgical area where the contents are

dispensed into individual sterile 3cc plastic syringes which are subsequently loaded into the fibrin glue applicator for spraying or line and dot application. Mixing the thrombin with the clotting and adhesive proteins forms the biological glue.

Both dispensing syringes 42 and 76 are stored at room temperature, or preferably stored at their optimal conditions: cryoprecipitate 76 being stored at room temperature and thrombin 42, stored in an ice bath at 1°C to 5°C. Please see figures 13 through 16.

Assume 9-10 ml of room temperature plasma is introduced into the reaction chamber 26. Other plasma volumes have utility. Please see figures 15 and 16. Add 1.0 ml of 75 mM calcium chloride ($CaCL_2$) and 2.0 ml of ethanol (ETOH) (i.e., ethanol taken from a 100% "stock" bottle and added to comprise 18.9% volume/unit volume or 15.02% ethanol weight/unit volume). Other ratios of reagent volume, comprising of ethanol (ETOH) (i.e., ethanol taken from a 100% "stock" bottle and a stock solution of 75 mM calcium chloride ($CaCL_2$)), to plasma volume have utility phase. Please see figures 13 and 14. The thrombin life span is shown to have been at least 300 minutes while its clotting time is at 2.98 seconds. An ethanol final concentration range between 8.0% and 20.0% (volume/unit volume), however, still has utility. Please see figure 11.

When the ethanol is at a final concentration of 18.9% volume/unit volume (as above) and the calcium chloride final concentration is 5.7 mM (1 ml taken from a 75 mM stock solution of calcium chloride), the thrombin lifespan also extends to at least 360 minutes while maintaining a clot time of 5.98 seconds when thrombin is stored at room temperature. Storing thrombin in optimal 1°C to 5°C ice bath typically maintains lot times of 2 to 3 seconds at 360 minutes. Calcium chloride stock solution concentrations ranging between 50 mM and 250 mM, however, have utility. Please see figure 12. The final concentrations range from 4.5mM to 23 mM.

Solutions such as saline, dilute $CaCL_2$ (e.g. 40mM to 125 mM $CaCL_2$) or even sterile water added to the thrombin can alter both the clotting time and life span of the thrombin. Assume an ethanol final concentration of 18.9% and a final calcium chloride concentration of 5.7 mM was used in the reaction chamber 26. When the thrombin has been diluted 1 to 1.5 with water, the clot time has been extended to just less than 30 seconds, and has a life span of up to 150 minutes.

Moreover, having thus described the invention, it should be apparent that numerous structural modifications and adaptations may be resorted to without departing from the scope and fair meaning of the instant invention as set forth hereinabove and as described hereinbelow by the claims.

Claims

We Claim:

Claim 1 - A method for generating autologous thrombin from a patient, the steps including:

obtaining a blood product from the patient;

sequestering plasma from the product;

enriching the prothrombin in a plasma fraction;

converting the prothrombin to thrombin, and

filtering particulate from the thrombin.

Claim 2 - The method of claim 1 further including the step of altering the clotting time.

Claim 3 - The method of claim 2 including adding ethanol to enrich the prothrombin in a plasma fraction.

Claim 4 - The method of claim 3 wherein the converting step includes adding $CaCL_2$.

Claim 5 - The method of claim 4 including centrifuging the blood product for obtaining plasma.

Claim 6 - The method of claim 2 wherein a predictable clotting time extension occurs through diluting the thrombin with any of the group consisting of saline, $CaCL_2$ solution and sterile water.

Claim 7 - The method of claim 6 including filtering the plasma by weight, size and protein binding.

Claim 8 - A method for producing fast clotting autologous thrombin which is stable for more than fifteen minutes, the steps including:

sequestering pro-thrombin from plasma and converting the pro-thrombin to thrombin.

Claim 9 - Autologous thrombin which provides fast clotting in less than five seconds for more than fifteen minutes.

Claim 10 - A composition for extracting thrombin from plasma consisting essentially of:

Plasma;

Ethanol (ETOH);

$CaCL_2$.

Claim 11 - The composition of claim 10 wherein ETOH is present at 18.9% and $CaCL_2$ is present at 23.0 mM both by volume in final concentration.

Claim 12 - The composition of claim 10 wherein ETOH is present at 18.9% and $CaCL_2$ is present at 5.7 mM both by volume in final concentration.

Claim 13 - The composition of claim 10 wherein ETOH is present at a range between 8% and 20% and $CaCL_2$ is present at a range between 4.5 mM and 23.0 mM both by volume in final concentration.

Claim 14 - A method for preparing thrombin comprising:

obtaining plasma;

adding ETOH and $CaCL_2$ to the plasma, forming a composition:

agitating the composition;

filtering the composition of particulate, thereby passing the thrombin through the filter.

Claim 15 - The method of claim 14 whereby subsequent to agitating the composition, incubating the composition for an amount of time greater than or equal to ten minutes.

Claim 16 - The method of claim 15 whereby prior to filtering the composition, re-agitating the composition.

Claim 17 - A device for preparing thrombin from plasma, comprising:

a reaction chamber having a solution of $CaCL_2$ and ETOH therein;

means for admitting plasma into said reaction chamber;

a thrombin receiving syringe coupled to said reaction chamber to receive the thrombin; and

a filter located between said reaction chamber and said thrombin receiving syringe.

Claim 18 - An autologous biological glue processing device, comprising, in combination:

a thrombin processing means,

a clotting and adhesive proteins processing means operatively coupled to said thrombin processing means,

means for receiving plasma via said operative coupling for subsequent conversion of the plasma to, respectively thrombin and clotting and adhesive proteins.

Claim 19 - A device for preparing thrombin from plasma, comprising:

a reaction chamber having ceramic beads or borosilicate glass therein;

means for admitting a reagent into said reaction chamber;

means for admitting plasma into said reaction chamber;

a thrombin receiving syringe coupled to said reaction chamber to receive the thrombin; and

a filter located between said reaction chamber and said thrombin receiving syringe.

Claim 20 - The device of claim 19 wherein the reagent includes $CaCL_2$ and ETOH solution.

Claim 21 - The method of claim 1 further including the step of contacting the plasma with glass beads.

Claim 22 - A composition for extracting thrombin from plasma consisting essentially of:

plasma;

ethanol (ETOH);

$CaCL_2$; and

glass beads.

Claim 23 - The composition of claim 22 wherein ETOH is present at 18.9% and $CaCL_2$ is present at 23.0 mM both by volume in final concentration.

Claim 24 - The composition of claim 22 wherein ETOH is present at 18.9% and $CaCL_2$ is present at 5.7 mM both by volume in final concentration.

Claim 25 - The composition of claim 22 wherein ETOH is present at a range between 8% and 20% and $CaCL_2$ is present at a range between 4.5 mM and 23.0 mM both by volume in final concentration.

Claim 26 - An apparatus to prepare thrombin from plasma consisting of:

a reacting chamber to accept $CaCL_2$ and ethanol means include injection of plasma into said reacting chamber;

a syringe to receive thrombin connected to said reacting chamber;

and a filter between said reacting chamber and syringe which is to receive thrombin.

Claim 27 - The apparatus of claim 26 further including glass beads beforehand.

AMENDED CLAIMS

[received by the International Bureau on 27 October 2000 (27.10.00); original claims 1.4-6, 8-14, 17, 18, 20 and 22-27 amended; new claims 28-53 added; other claims unchanged (7 pages)]

Claim 1 - A method for generating autologous thrombin from a patient, the steps including:

obtaining a blood product from the patient;

sequestering unadulterated plasma from the product;

enriching the prothrombin in an unadulterated plasma fraction;

converting the prothrombin to thrombin, and

filtering particulate from the thrombin.

Claim 2 - The method of claim 1 further including the step of altering the clotting time.

Claim 3 - The method of claim 2 including adding ethanol to enrich the prothrombin in a plasma fraction.

Claim 4 - The method of claim 3 wherein the converting step includes adding a source of calcium ions.

Claim 5 - The method of claim 4 including centrifuging the blood product for obtaining unadulterated plasma.

Claim 6 - The method of claim 2 wherein a predictable clotting time extension occurs through diluting the thrombin with any of the group consisting of saline, $CaCl_2$ solution and sterile water.

Claim 7 - The method of claim 6 including filtering the plasma by weight, size and protein binding.

Claim 8 - A method for producing fast clotting autologous thrombin which is stable for more than fifteen minutes, the steps including:

sequestering prothrombin from unadulterated plasma and converting the prothrombin to thrombin.

Claim 9 - Autologous thrombin which provides fast clotting in less than five seconds and is stable for more than fifteen minutes.

Claim 10 - A composition for extracting thrombin from plasma consisting essentially of:

unadulterated Plasma;

Ethanol (ETOH);

$CaCl_2$.

Claim 11 - The composition of claim 10 wherein ETOH is present at 18.9% and $CaCl_2$ is present at 23.0 mM both by volume in final concentration.

Claim 12 - The composition of claim 10 wherein ETOH is present at 18.9% and $CaCl_2$ is present at 5.7 mM both by volume in final concentration.

Claim 13 - The composition of claim 10 wherein ETOH is present at a range between 8% and 20% and $CaCl_2$ is present at a range between 4.5 mM and 23.0 m M both by volume in final concentration.

Claim 14 - A method for preparing thrombin comprising:

obtaining unadulterated plasma;

adding ETOH and $CaCl_2$ to the unadulterated plasma, forming a composition:

agitating the composition;

filtering the composition of particulate, thereby passing the thrombin through the filter.

Claim 15 - The method of claim 14 whereby subsequent to agitating the composition, incubating the composition for an amount of time greater than or equal to ten minutes.

Claim 16 - The method of claim 15 whereby prior to filtering the composition, re-agitating the composition.

Claim 17 - A device for preparing thrombin from plasma, comprising:

a reaction chamber having a solution of $CaCl_2$ and ETOH therein;

means for admitting unadulterated plasma into said reaction chamber;

a thrombin receiving syringe coupled to said reaction chamber to receive the thrombin; and

a filter located between said reaction chamber and said thrombin receiving syringe.

Claim 18 - A single donor biological glue processing device, comprising, in combination:

a thrombin processing means,

a clotting and adhesive proteins processing means operatively coupled to said thrombin processing means,

means for receiving plasma via said operative coupling for subsequent conversion of the plasma to, respectively thrombin in said thrombin processing means and clotting and adhesive proteins in said clotting and adhesive proteins processing means.

Claim 19 - A device for preparing thrombin from plasma, comprising:

a reaction chamber having ceramic beads or borosilicate glass therein;

means for admitting a reagent into said reaction chamber;

means for admitting plasma into said reaction chamber;

a thrombin receiving syringe coupled to said reaction chamber to receive the thrombin; and

a filter located between said reaction chamber and said thrombin receiving syringe.

Claim 20 - The device of claim 19 wherein the reagent includes $CaCl_2$ and ETOH solution.

Claim 21 - The method of claim 1 further including the step of contacting the plasma with glass beads.

Claim 22 - A composition for extracting thrombin from plasma consisting essentially of:

plasma;

ethanol (ETOH);

$CaCl_2$; and

glass beads.

Claim 23 - The composition of claim 22 wherein ETOH is present at 18.9% and $CaCl_2$ is present at 23.0 mM both by volume in final concentration.

Claim 24 - The composition of claim 22 wherein ETOH is present at 18.9% and $CaCl_2$ is present at 5.7 mM both by volume in final concentration.

Claim 25 - The composition of claim 22 wherein ETOH is present at a range between 8% and 20% and $CaCl_2$ is present at a range between 4.5 mM and 23.0 mM both by volume in final concentration.

Claim 26 - An apparatus to prepare thrombin from plasma consisting of:

a reacting chamber to accept $CaCl_2$ and ethanol, and means for delivery of plasma into said reacting chamber;

a syringe connected to said reacting chamber to receive thrombin from said reacting chamber;

and a filter between said reacting chamber and syringe which is to receive thrombin.

Claim 27 - The apparatus of claim 26 further including glass beads in said reacting chamber.

Claim 28 - A method for generating and then dispensing thrombin, the steps including:

taking whole blood from one person,

sequestering prothrombin from the whole blood,

converting the prothrombin to thrombin,

loading the thrombin into a syringe,

and then applying the thrombin onto an area to stem blood flow.

Claim 29 - The method of claim 28 including loading clotting proteins into another syringe and dispensing the clotting proteins concurrently with the thrombin.

Claim 30 - A method for generating thrombin from one person, the steps including:

sequestering prothrombin from plasma taken from the person,

converting the prothrombin to thrombin and removing particulate material therefrom.

Claim 31 - The method of claim 30 further including diluting the thrombin in order to alter clotting time of the thrombin.

Claim 32 - The method of claim 31 including adding a source of calcium ions to alter the clotting time.

Claim 33 - The method of claim 32 including adding $CaCl_2$ to alter the clotting time.

Claim 34 - The method of claim 31 including adding saline to alter the clotting time.

Claim 35 - The method of claim 31 including adding sterile water to alter the clotting time.

Claim 36 - The method of claim 2 wherein the step of altering the clotting time includes adding a source of calcium ions.

Claim 37 - The method of claim 2 wherein the step of altering the clotting time includes adding $CaCl_2$.

Claim 38 - The method of claim 2 wherein the step of altering the clotting time includes adding saline.

Claim 39 - The method of claim 2 wherein the step of altering the clotting time includes adding sterile water.

Claim 40 - A method for generating thrombin from one person, the steps including:

taking whole blood from the one person,

obtaining plasma from the whole blood,

converting prothrombin in the plasma to thrombin and

sequestering the thrombin from the plasma.

Claim 41 - The method of claim 40 including altering the clotting time of the thrombin to clot in less than five seconds.

Claim 42 - The method of claim 41 wherein the step of altering the clotting time includes adding a source of calcium ions.

Claim 43 - The method of claim 42 wherein the step of altering the clotting time includes adding $CaCl_2$.

Claim 44 - The method of claim 41 wherein the step of altering the clotting time includes adding saline.

Claim 45 - The method of claim 41 wherein the step of altering the clotting time includes adding sterile water.

Claim 46 - The method of claim 40 including making the thrombin stable for more than fifteen minutes.

Claim 47 - The method of claim 46 including adding a source of calcium ions to alter the clotting time.

Claim 48 - The method of claim 47 including adding $CaCl_2$ to alter the slotting time.

Claim 49 - The method of claim 46 including adding saline to alter the clotting time.

Claim 50 - The method of claim 46 including adding sterile water to alter the clotting time.

Claim 51 - The method of claim 1, the steps including adding the reagents ETOH and $CaCl_2$ to the plasma followed by agitation to form the thrombin.

Claim 52 - The method of claim 51 further including:

taking whole blood from one person,

sequestering prothrombin from the whole blood,

converting the prothrombin to thrombin,

loading the thrombin into a syringe,

and then applying the thrombin onto an area to stem blood flow.

Claim 53 - The device of claim 18 including a thrombin syringe coupled to said thrombin processing means to receive thrombin therefrom, said thrombin syringe initially ensconced in a bag, and

a clotting and adhesive protein syringe coupled to said clotting and adhesive protein processing means to receive clotting and adhesive proteins therefrom, said clotting and adhesive protein syringe initially ensconced in a bag.

10/009417

PATENT COOPERATION TREATY

REC'D 27 NOV 2001

WIPO PCT

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
80088-pct		
International application No.	International filing date (day/month/year)	Priority date (day/month/year)
PCT/US00/15955	07 JUNE 2000	04 JUNE 1999

International Patent Classification (IPC) or national classification and IPC
Please See Supplemental Sheet

Applicant
THERMOGENESIS CORP

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets.

   [X] This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 25 sheets.

3. This report contains indications relating to the following items:

   I [X] Basis of the report

   II [ ] Priority

   III [ ] Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV [ ] Lack of unity of invention

   V [X] Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI [ ] Certain documents cited

   VII [ ] Certain defects in the international application

   VIII [ ] Certain observations on the international application

CORRECTED VERSION

Date of submission of the demand	Date of completion of this report
04 JANUARY 2001	27 JULY 2001
Name and mailing address of the IPEA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	RALPH GILLENWATER
Facsimile No. 703-305-3230	Telephone No. 703-308-0651

Form PCT/IPEA/409 (cover sheet) (July 1998) *

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US00/11865

I. Basis of the report

1 With regard to the **elements** of the international application: *

- [ ] the international application as originally filed
- [X] the description
  - pages: (See Attached) , as originally filed
  - pages: , filed with the demand
  - pages: , filed with the letter of
- [X] the claims
  - pages: (See Attached) , as originally filed
  - pages: , as amended (together with any statement) under Article 19
  - pages: , filed with the demand
  - pages: , filed with the letter of
- [X] the drawings
  - pages: (See Attached) , as originally filed
  - pages: , filed with the demand
  - pages: , filed with the letter of
- [X] the sequence listing part of the description
  - pages: (See Attached) , as originally filed
  - pages: , filed with the demand
  - pages: , filed with the letter of

2 With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language: which is:

- [ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b))
- [ ] the language of publication of the international application (under Rule 48.3(b))
- [ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3)

3 With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- [ ] contained in the international application in printed form
- [ ] filed together with the international application in computer readable form
- [ ] furnished subsequently to this Authority in written form
- [ ] furnished subsequently to this Authority in computer readable form
- [ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished
- [ ] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4 [X] The amendments have resulted in the cancellation of:

- [X] the description, pages: NONE
- [X] the claims, Nos.: NONE
- [X] the drawings, sheets/fig: NONE

5 [ ] This report has been drawn as if some of the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	Please See supplemental sheet	YES
	Claims	Please See supplemental sheet	NO
Inventive Step (IS)	Claims	Please See supplemental sheet	YES
	Claims	Please See supplemental sheet	NO
Industrial Applicability (IA)	Claims	Please See supplemental sheet	YES
	Claims	Please See supplemental sheet	NO

2. citations and explanations (Rule 70.7)

Claims 1, 2, 4-56, 58-72 lack an inventive step under PCT Article 33(3) as being obvious over each of Cederholm-Williams and Hirsh

Cederholm-Williams 5,797,798 entitled "Method of Use of Autologous Thrombin Blood Fraction in a Cell Culture with Keratinocytes" teaches in column 2 last 2 paragraphs, a thrombin blood fraction can be prepared in about one or two hours from whole blood and used in an autologous medical procedure. In column 4 lines 25-36, the thrombin can be stored for a period of months or days. In column 5 first full paragraph, whole blood is obtained, plasma is fractionated by any separation technique. In column 5 last 2 paragraphs saline buffers and other compounds may be added. Calcium chloride is added to convert prothrombin to thrombin. In column 6 line 54 the thrombin can be prepared in only about 45 minutes. In column 9 last full paragraph, a syringe is shown.

Hirsh 5,643,192 entitled "Autologous Fibrin Glue and Methods for its Preparation and Use" teaches in column 3 last paragraph bridging to column 4 separating the fibrinogen from thrombin adding calcium chloride, filtering to produce thrombin. The thrombin can be applied to a treatment site in a patient with a syringe. Autologous donations are discussed in column 3.

The claims differ from the above references in that they recite ethanol is an additive to enrich prothrombin in a plasma fraction. On page 28 of the present specification various ethanol concentrations and the resultant thrombin lifespan are discussed. However no amounts or concentrations of ethanol are claimed nor any thrombin lifespans.

As the claims are written it is difficult for the examiner to determine any point of novelty. See Page 28 "Solutions such as saline, dilute CaCl2 or even water added to the thrombin can alter both the clotting time and life span of the thrombin."

It would have been obvious to one of ordinary skill in the art at the time the invention was made to employ any desired physiologically acceptable solvent to dilute the thrombin product. Continued on Supplemental Sheet

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient

Continuation of: Boxes I - VIII

CLASSIFICATION
The International Patent Classification (IPC) and or the National classification are as listed below
IPC(7): A61K 38/48, B01L 11/00, C12N 9/74, C12Q 1/56, G01N 33/00 and US Cl.: 422/73, 101, 424/94.64, 435/13, 214

I BASIS OF REPORT

This report has been drawn on the basis of the description,
page(s) 1, as originally filed
page(s) 2-18, filed with the demand
and additional amendments
NONE

This report has been drawn on the basis of the claims,
page(s) none, as originally filed
page(s) none, as amended under Article 19
page(s)NONE, filed with the demand
and additional amendments
Pages 19-26 filed with the letter of 22 June 2001

This report has been drawn on the basis of the drawings,
page(s) 1-15, as originally filed
page(s) none, filed with the demand
and additional amendments
none

This report has been drawn on the basis of the sequence listing part of the description,
page(s) NONE, as originally filed
pages(s) NONE, filed with the demand,
and additional amendments:
NONE

V 1 REASONED STATEMENTS
The report as to Novelty was positive (YES) with respect to claims 3, 10-17, 20, 22-25, 51, 52
The report as to Novelty was negative (NO) with respect to claims 1, 2, 4-9, 18-19, 21, 26-50, 53
The report as to Inventive Step was positive (YES) with respect to claims none.
The report as to Inventive Step was negative (NO) with respect to claims 1-53
The report as to Industrial Applicability was positive (YES) with respect to claims 1-53
The report as to Industrial Applicability was negative (NO) with respect to claims none

V 2 REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued)
in view of the above references. Further, it is not seen that ethanol enriches prothrombin in a plasma fraction. To select any well known diluent for its known function would have been obvious in view of the above references. Altering the clotting time by diluting the thrombin fraction or by using a desired quantity at a given concentration is taught by the references

NEW CITATIONS
NONE